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Isolation of mouse N-CAM-related cDNA: detection and cloning using monoclonal antibodies

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Clones coding for the mouse neural cell adhesion molecule (N-CAM) were isolated from a cDNA library prepared in the expression vector λ gt 11 from mRNA extracted from a mouse neuroblastoma cell line. This library was screened with two anti-N-CAM monoclonal antibodies directed against different sites on the molecule and with rabbit anti-N-CAM serum. Two clones were identified with the first monoclonal antibody, three with the second one, none reacted with both. The relevance of these cDNA clones to N-CAM was confirmed by several observations. First, cDNA sequences detected with one monoclonal antibody cross-hybridized with those identified by the other antibody. Second, the different fusion proteins all bound the rabbit serum in addition to one monoclonal antibody. Finally, the probes hybridized to discrete mRNA species of sufficient lengths to code for the very large N-CAM polypeptides in RNA preparations from N-CAM-expressing, but not from N-CAM-negative cells. An additional mRNA species not seen in embryonic brain was expressed in adult mouse brain. Genomic blot experiments indicated that sequences corresponding to one of our probes are present only a few times in the mouse genome.

Key words: N-CAM/expression cloning/monoclonal antibodies/mouse brain

Introduction

Adhesive interactions between cell surfaces are of central importance to the assembly of cells into tissues and organs during ontogenesis. Of the vertebrate adhesion molecules likely to be involved in morphogenetic processes, the cell surface glycoproteins named N-CAM (neural cell adhesion molecules) have been studied most extensively. Initially identified in chick retina and brain (Rutishauser *et al.*, 1976) N-CAMs occur also on chick muscle cells (Grumet *et al.*, 1982) and transiently on other tissues of the chick embryo (Thiery *et al.*, 1982). Very similar molecules are present in other vertebrate species (Hirn *et al.*, 1981; Chuong *et al.*, 1982; Gennarini *et al.*, 1984a; Rasmussen *et al.*, 1982). The adhesive properties of the N-CAMs are consistent with a role as a ligand in the formation of cell-cell bonds (for reviews, see Rutishauser, 1983; Edelman *et al.*, 1983; Goridis *et al.*, 1983).

Different forms of N-CAM that are selectively expressed in different nervous tisses and during development have been identified. A highly sialylated form is prevalent in more immature nervous tissue and is gradually converted into a less sialylated adult form during development, and these changes can be correlated with changes in the binding properties of the molecules (Hoffman and Edelman, 1983; Sadoul *et al.*, 1983). The polypeptide part of the N-CAMs is also heterogeneous. In the mouse, three structurally related proteins with mol. wts. of 180, 140 and 120 K have been identified (Chuong *et al.*, 1982; Rougon *et al.*, 1982). They appear to differ mainly in the length of their cytoplasmic extensions (Gennarini *et al.*, 1984b), but each of these mol. wt. classes could still consist of several closely similar but distinct polypeptides. The latter possibility gains some support from the finding that N-CAMs from different brain regions can be distinguished serologically (Chuong and Edelman, 1984).

Studies of N-CAM biosynthesis by neural cells (Lyles *et al.*, 1984) suggest that the 180- and 140-K molecules are synthesized from different mRNAs, and our own unpublished work indicates that the same may be true for the 120-K chain, but it is not known to what extent multiple genes, multiple mRNA species or post-translational modifications contribute to structural differences between N-CAM polypeptides. It is also not known whether the N-CAMs are structurally related to other cell adhesion molecules of similar size as might be inferred from some serological data (Grumet *et al.*, 1984; Kruse *et al.*, 1984). Answers to these questions are most readily obtained by recombinant DNA methods. Recently, two cDNA clones for chicken N-CAM have been isolated from embryonic brain mRNA enriched by immunoprecipitation (Murray *et al.*, 1984). We have adopted a different strategy. Mouse N-CAM cDNA sequences were isolated by screening a cDNA library constructed in a phage expression vector with monoclonal antibodies (mAbs). First described by Young and Davis (1983a), the λ gt 11 expression system has been successfully used to clone cDNAs with polyclonal antibody probes (Schwarzbauer *et al.*, 1983; Leytus *et al.*, 1984). It has been argued that mAbs are not suitable for screening expression libraries because of fortuitous cross-reactions (Young and Davis, 1983b). As shown here screening with two mAbs, which recognize different sites on the same molecule, can successfully be used to isolate and to confirm the identity of N-CAM-derived cDNA clones.

Results

Isolation of N-CAM cDNA clones

Poly(A)$^+$ RNA was isolated from membrane-bound polysomes of the N-CAM-expressing N2A neuroblastoma line and the cDNA cloned into the *Eco*RI site of the phage expression vector λ gt 11. In this vector, the cDNA is inserted into the *lacZ* gene and, when in the correct reading frame and orientation, can be expressed by the bacteria as a fusion protein (Young and Davis, 1983a). We screened our library with the mAbs H28 and P61 which react with distinct sites on mouse N-CAM molecules (Gennarini *et al.*, 1984a). Screening of $\sim 10^5$ recombinants identified four clones which in successive rounds of rescreening were consistently positive. Clones M1 and M2 were detected by H28, clones M3 and M4 by P61. Screening of another 1.5 x 10^5 plaques with our rabbit anti-N-CAM serum yielded only one positive

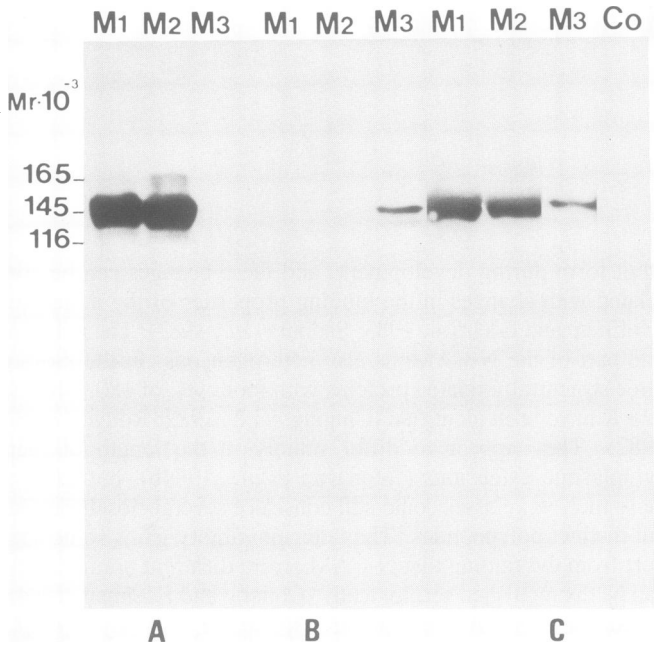

Fig. 1. Analysis of fusion proteins. Bacterial lysates were prepared from the lysogens induced in *E. coli* Y1089 with M1, M2 and M3. The proteins (300 μg applied to each lane) were separated on 6.7% polyacrylamide gels, transferred to nitrocellulose and probed with mAbs H28 (**panel A**), P61 (**panel B**) and rabbit anti-N-CAM serum (**panel C**). The mAbs were used in the form of 1/25 diluted hybridoma supernatants and revealed by [^{125}I]iodinated rabbit anti-rat Ig, the rabbit serum diluted 1/10^3 and revealed by [^{125}I]iodinated protein A. A lysate of Y1089 infected with non-recombinant phage was used as control (Co). The position of *E. coli* β-galactosidase run in an adjacent lane and revealed by Coomassie blue staining is indicated at the left (116 K) as well as the calculated mol. wts. for the band in M1 (~145 K) and the slower migrating component in M2 (~165 K) lyates. As seen in **panel C**, the fusion protein from M3 lysates runs just behind the M2 band corresponding to a mol. wt. of ~150 K. A 165-K band is revealed in lane M2 of **panel C** after longer exposure.

clone (H1). This recombinant was also detected by P61. Considerably stronger signals were obtained with the mAbs than with the rabbit serum. Most likely, the polyclonal sera contain antibodies against many different determinants and the ones recognizing the product of a particular cDNA are less represented in the serum than in a mAb preparation.

The inserts of M1, M2 and M3 were single *Eco*RI fragments of 0.58, 1.35 and 0.70 kb, respectively. Clone M4 contained two (0.66 and 0.20 kb) and H1 three (0.75, 0.59 and 0.20 kb) inserts and were not characterized further. To find out whether fusion proteins of the corresponding size were indeed recognized by our antibodies, lysogens of M1, M2 and M3 were prepared, induced with isopropyl thiogalactoside (IPTG), and the lysates analysed by Western blotting (Figure 1). As expected, H28 bound to the M1 and M2, P61 to the M3 fusion proteins. Rabbit anti-N-CAM serum produced a strong signal with all three of them. The mol. wt. of the fusion proteins detected in M1 and M3 lysates were ~30 and 35 K bigger than β-galactosidase, respectively. This corresponds in size to the lengths of the inserts. Two polypeptides were revealed in M2 lysates, the larger of which had a mol. wt. of 165 K as would be expected for a 1.35-kb insert. The second, much stronger band was probably a proteolytic breakdown product, since *Escherichia coli* tends to degrade very long fusion proteins (Kemp *et al.*, 1983). These results demonstrated that N-CAM antigenic determinants were present in the fusion proteins and that the three inserts contained open reading frames over most of their lengths.

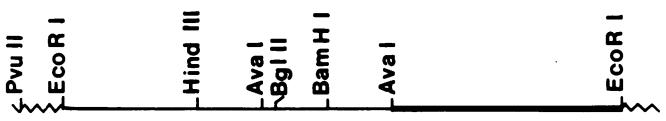

Fig. 2. Partial restriction map of the 1.35-kb *Eco*RI fragment from pM2.2. The *Eco*RI sites delineate the cDNA insert. The *Pvu*II site of pBR328 is indicated for orientation. The thick line designates the fragment to which the inserts of pM1.3 and pM3.7 hybridize. The following enzymes failed to cut the pM2.2 insert: *Acc*I, *Bcl*I, *Hinc*III, *Hpa*I, *Kpn*I, *Pst*I, *Pvu*I, *Pvu*II. None of them nor the ones indicated in the Figure cut the pM1.3 or pM3.7 inserts.

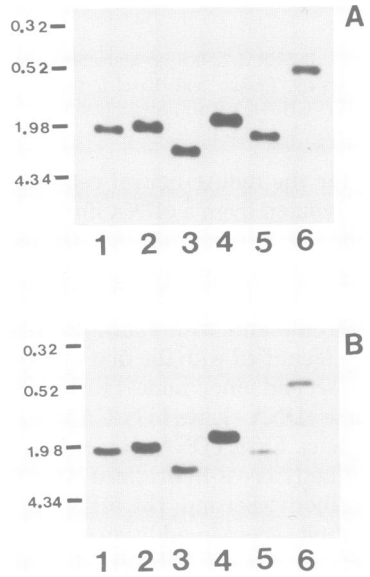

Fig. 3. Blot hybridization of the nick-translated inserts from pM1.3 (**A**) and pM3.7 (**B**) to restriction digests of plasmid pM2.2. Single digestions were with *Bam*I (**lane 1**), *Ava*I (**lane 3**), *Hind*III (**lane 5**), double digestions with *Bam*HI plus *Ava*I (**lane 2**), *Hind*III plus *Ava*I (**lane 4**) and *Bgl*II plus *Eco*RI (**lane 6**). The fragments were blotted to nitrocellulose and hybridized wtih 10^5 d.p.m./ml of the nick-translated inserts. The autoradiograph shown in **A** was exposed for 40 min, the one shown in **B** for 4 h. The position and sizes of mol. wt. markers are indicated. The following restriction fragments were revealed in the different lanes by ethidium bromide staining (with the hybridizing fragments underlined): **lane 1**, 4.0 and 2.3 kb; **lane 2**, 3.8, 2.1 and 0.32 kb; **lane 3**, 3.2, 2.8 and 0.32 kb; **lane 4**, 4.0, 1.8 and 0.32 kb, **lane 5**, 4.0 and 2.3 kb; **lane 6**, 4.9, 0.85 and 0.52 kb.

Different clones contain cross-hybridizing inserts

The cDNAs of M1, M2 and M3 were subcloned into the *Eco*RI site of pBR328 to generate clones pM1.3, pM2.2 and pM3.7. The gel purified *Eco*RI fragments were used as hybridization probes against the original phages. Probes 1.3 and 2.2 reacted strongly with both M2 and M1; probe 3.7 with M3 and M4 (not shown). This was to be expected since the strongly cross-hybridizing clones had been identified by the same mAb. However, mAbs may recognize not only similar sequences on different proteins but also similar epitopes on proteins with unrelated primary structure. Cross-reaction at the cDNA level proves that the inserts share common sequences. The insert of pM2.2 also gave a positive, albeit much weaker signal with phage M3. Cross-hybridization between the inserts of M1 and M3 were very weak and of doubtful significance (data not shown).

The homologies implied by hybridization to the phage DNA were further explored by restriction analysis and by blot hybridization using nick-translated inserts from pM1.3 and 3.7 against restriction digests of pM2.2. A partial restriction map of

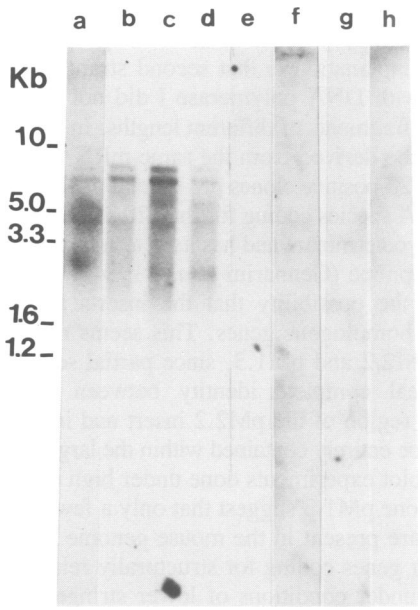

Fig. 4. Blot hybridization of nick-translated probe pM1.3 to RNA from N-CAM-positive and -negative cells and tissues. **Lane a**, 5 μg poly(A)$^+$ RNA from N2A cells; **lane b**, 10 μg poly(A)$^+$ RNA from embryonic day 17 mouse brain; **lane c**, 10 μg poly(A)$^+$ RNA from post-natal day 2 mouse brain; **land d**, 10 μg poly(A)$^+$ RNA from post-natal day 30 mouse brain; **lane e**, 15 μg poly(A)$^+$ RNA from a B cell hybridoma; **lane f**, 10 μg poly(A)$^+$ RNA from adult mouse liver; **lane g**, 10 μg poly(A)$^+$ RNA from LMTK$^-$ mouse fibroblasts; **lane h**, 10 μg poly(A)$^+$ RNA from adult mouse liver. The filters were hybridized with 0.1 μg of labeled probe (4 x 10^7 d.p.m.) in 5 x SSC, 0.1% Ficoll 400, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 50 μg/ml tRNA, 100 μg/ml denatured salmon sperm DNA, 0.1% SDS, 50 mM phosphate buffer (pH 6.8), 50% formamide and 10% dextran sulfate for 20 h at 42°C. They were washed at 65°C for 20 min each twice with 2 x SSC, 0.2% SDS and once with 0.2 x SSC. Exposure time was 1 week at −70°C in the presence of sensitizing screens. DNA restriction fragments were used as markers, their size is given in kb. They were treated in the same way as the RNA samples, run on the same gel and revealed by hybridization to the labeled probes.

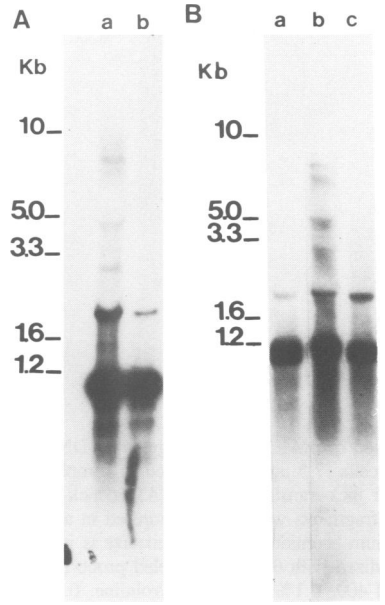

Fig. 5. Blot hybridization of nick-translated probes pM2.2 (**panel A**) and pM3.7 (**panel B**) to RNA from N-CAM-positive and -negative cells and tissues. **Panel A: lane a**, total cytoplasmic RNA from N2A cells; **lane b**, total cytoplasmic RNA from LMTK$^-$ cells. **Panel B: lane a**, total cytoplasmic RNA from LMTK$^-$ cells; **lane b**, total cellular RNA from neonatal mouse brain; **lane c**, total cellular RNA from adult mouse liver. 20 μg RNA was applied to each lane. The two highest mol. wt. bands are 7.2 and 6.3 kb in length. Exposure time was for 3 days; due to the predominance of the 1.2-kb RNA species longer exposure times would have obscured most of the gel. Size markers and hybridization conditions were the same as indicated in Figure 4 legend.

pM2.2 is presented in Figure 2. Clones pM1.3 and pM3.7 lacked all of the restriction sites mapped in the pM2.2 insert (data not shown). Hybridization of the pM1.3 insert to restricted pM2.2 DNA revealed that the zone of homology was limited to the larger *Ava*I-*Eco*RI fragment (thick line in Figure 2) as pM1.3 hybridized only to the *Hind*III, *Bam*HI, *Ava*I and *Bgl*II/*Eco*RI fragments which contained this part of the pM2.2 insert (Figure 3A). The insert of pM3.7 hybridized to the same restriction fragments, but the signal was considerably less intense. In fact, the autoradiograph shown in Figure 3B was exposed six times longer than the one presented in Figure 3A. This indicated incomplete homology or hybridization *via* a short stretch of nucleotides.

RNA blot anlaysis

Total and poly(A)$^+$ RNA from neonatal or adult brain and adult liver and cytoplasmic and poly(A)$^+$ RNA from N2A, LMTK$^-$ and B hybridoma cells were analyzed by hybridization with the nick-translated plasmids pM1.3, pM2.2 and pM3.7. Plasmid pM1.3 revealed mRNA bands only in extracts from N-CAM-expressing tissues and cells but not from N-CAM-negative cells (Figure 4). Bands of ~7.2, 6.3, 6.0, 4.8 and 2.5 kb were detected in adult brain. Considerable changes in the absolute and relative intensities of these RNA bands were observed throughout brain development. First, increased expression of the 7.2- and 6.3-kb bands was observed in neonatal brain. Second, the 6.0- and 2.5-kb bands hardly if at all detectable in embryonic brain, became faintly visible at birth and as intense as the two highest mol. wt. classes in the adult. The 4.8-kb mRNA species, by contrast, was present at almost equal intensity throughout development.

Although with our present data, we cannot exclude that the lower mol. wt. species are breakdown products of the highest mol. wt. class, their presence as rather discrete bands and the developmental changes in their relative intensities strongly argue against this possibility. Since the mRNA sequences to which pM1.3 hybridized were only detected in N-CAM-expressing tissues and cells, this probe may be speciic for sequences coding for N-CAM.

In contrast to pM1.3, clones pM2.2 and pM3.7 gave strong signals with 1.2- and 2.0-kb RNA bands equally present in N-CAM-positive and negative cells (Figure 5). Larger RNA species were also revealed and they appeared specific for the N-CAM-expressing cells. Both probes bound to RNA bands of identical size again underlining the homologies between the cDNAs. The two neural cell-specific RNA species of highest mol. wt. identified by pM2.2 and pM3.7 had the same size as those detected by pM1.3.

Hybridization to genomic DNA

High mol. wt. DNA from N2A cells was digested with *Eco*RI, electrophoresed on 0.8% agarose gels, transferred to nitrocellulose and hybridized to nick-translated pM1.3. As seen in Figure 6 only two bands were detected. Fragments of the same size were revealed by probe pM3.7 (not shown).

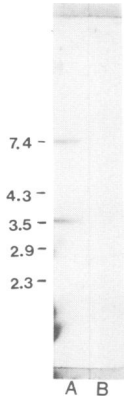

Fig. 6. Hybidization of pM1.3 to mouse genomic DNA. High mol. wt. DNA from N2A cells (7.5 μg) was digested to completion with *Eco*RI and probed with either nick-translated pM1.3 (**A**) or nick-translated pBR328 (**B**). Phage restriction fragments were electrophoresed in adjacent lanes and revealed by ethidium bromide staining, their size is indicated in kb. The filters were hybridized with 0.1 μg of labeled probe (3 x 10^7 d.p.m.) in 5 x SSC, 0.1% Ficoll 400, 0.1% polyvinylpyrrolidine, 0.1% bovine serum albumin, 0.1% SDS, 5 mM EDTA and 150 μg/ml denatured salmon sperm DNA for 24 h at 68°C. They were washed at room temperature for 15 min each twice with 3 x SSC, 0.1% SDS and twice with 0.1 x SSC, 0.1% SDS. Exposure time was 4 days at −70°C in the presence of sensitizing screens.

Discussion

We have used monoclonal antibodies to isolate cDNA clones coding for mouse N-CAM by the antibody screening method first described by Young and Davis (1983a). Monoclonal antibodies can theoretically recognize expression products unrelated to the protein under study since single epitopes can be shared by different proteins. This risk was minimized by using two mAbs reacting with different sites on N-CAM polypeptides. In fact, strong evidence that our clones contain sequences coding for mouse N-CAM is provided by cross-hybridization not only between recombinants recognized by the same mAb but also between two clones recognized by different mAbs. Moreover, clones pM1.3 and pM2.2, detected by H28, and clone pM3.7, detected by P61, hybridized to high mol. wt. RNA of the same size. Finally, the products of these three recombinant phages bound not only one or the other of the mAbs but also our rabbit anti-N-CAM antibodies. The conclusion that the clones contain sequences complementary to mouse N-CAM mRNA was further substantiated by the observation that clone pM1.3 hybridized only to RNA species expressed in N-CAM synthesizing tissues and cells. Definite proof that the clones are coding for N-CAM polypeptides must await matching of cDNA and protein sequences. However, sufficient protein sequences for N-CAM are not yet available.

One advantage of screening an expression library with antibodies is that cDNAs containing protein coding sequences are selected. Our data on the expression of the fusion proteins by *E. coli* show that all cDNAs contain, at most, short non-coding regions. Furthermore, our mapping of the epitopes for the two mAbs on the protein (Gennarini *et al.*, 1984b) yields information on the approximate location of the sequences recognized. The site to which H28 binds has been localized to a segment around 90 K from the N terminus; the site reacting with P61 closer to the C terminus at the cytoplasmic side of the plasma membrane. Clone pM1.3 and 2.2, identified with H28, should thus contain sequences located downstream from those contained in pM3.7 which were detected by P61. It may seem surprising that sequences rather far from the 3′ end have been isolated from a cDNA library constructed *via* oligo d(T) priming. The most trivial explanation is that second strand cDNA synthesis carried out with DNA polymerase I did not go to completion thus creating fragments of different lengths. In this case, all three clones could be derived from the same mRNA. It is also possible that the H28-positive clones pM1.3 and pM2.2 are transcribed from a mRNA species coding for the 120 K N-CAM protein that lacks the P61 determinant and has its C terminus just downstream of the H28 epitope (Gennarini *et al.*, 1984b). Finally, we cannot exclude the possibility that the inserts are derived from distinct, but homologous genes. This seems not to be the case for clones pM2.2 and pM1.3, since partial sequence data (not shown) reveal complete identity between pM1.3 and the homologous region of the pM2.2 insert and indicate that clone pM1.3 may be entirely contained within the larger pM2.2 cDNA.

Genomic blot experiments done under high stringency conditions with clone pM1.3 suggest that only a few copies of the N-CAM gene are present in the mouse genome. It remains to be seen whether genes coding for structurally related proteins will be detected under conditions of lower stringency.

Recently, two putative cDNA clones for chicken N-CAM have been isolated (Murray *et al.*, 1984). The two mRNAs recognized by these clones in embryonic chick brain have approximately the same mol. wt. as the two largest bands revealed by our inserts. However, we detect additional bands in mouse brain. Species differences may account for this discrepancy as mouse brain N-CAM is known to include a 120-K chain which is not present in the chick (Chuong *et al.*, 1982; Rougon *et al.*, 1982; Rothbard *et al.*, 1982). However, Murray *et al.* (1984) have analysed only embryonic chick brain and it is possible that additional RNA species are present at later stages of development. The 7.2, 6.3, 6.0 and 4.8 kb mRNAs we detect have the coding potential necessary for the three N-CAM polypetides of different mol. wts. identified in mouse brain. As judged from its intensity, the 1.2-kb band found in all cell types and revealed by probes pM2.2 and pM3.7 should represent a rather abundant mRNA species. It may hybridize to a short stretch of non-coding region or else code for a protein which shares common sequences with N-CAM. Changes in the relative intensities of the mRNA species detected with clone pM1.3 were observed during brain development. Work is now in progress to characterize the different RNA molecules identified with our N-CAM cDNA clones.

Materials and methods

Isolation of RNA

Mouse N2A neuroblastoma cells were cultured as monolayers in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. A microsomal fraction enriched in membrane-bound polysomes was prepared from the cells and RNA extracted as described (Burke and Warren, 1984) except that 10 mM vanadyl ribonucleoside complex (BRL) was added to the buffers. Poly(A)$^+$ RNA was enriched by two cycles of chromatography on oligo(dT)-cellulose.

For use in Northern blotting experiments, cytoplasmic RNA was extracted from N2A cells and from LMTK$^-$ mouse fibroblasts according to Favaloro *et al.*, (1980) and total cellular RNA from mouse liver and brain by the guanidinium-cesium chloride method (Maniatis *et al.*, 1982). Poly(A)$^+$ RNA was selected by the messenger affinity paper (Medac, Hamburg) method (Werner *et al.*, 1984). Poly(A)$^+$ RNA from the B cell hybridoma G5 was a kind gift of P.Ollier.

cDNA synthesis and cloning

Reverse transcription conditions were 0.1 mg/ml RNA, 50 mM Tris-HCl (pH 8.3), 6 mM $MgCl_2$, 40 mM KCl, 0.1 mg/ml oligo(dT) (P.-L. Biochemicals), 1500 U/ml RNasin (Biotec), 0.5 mM of each dATP, dGTP, dTTP, 0.25 mM, 0.4 mCi/ml [α-^{32}P]dCTP and 72 U avian myeloblastosis virus reverse transcriptase (Stehelin). Incubation was for 60 min at 42°C. The reaction mixture was boiled for 3 min, diluted four times with H_2O and second strand synthesis

allowed to proceed for 16 h at 12°C in the presence of 300 U/ml DNA polymerase I (Stehelin), the aforesaid concentrations of unlabeled and radioactive nucleotide triphosphates, 50 mM Hepes (pH 6.9), 80 mM KCl, 27 mM $MgCl_2$, 1 mM DTT and 50 μg/ml bovine serum albumin. The double-stranded cDNA was treated with S1 nuclease (Stehelin), T4 DNA polymerase (BRL)-treated and ligated to *Eco*RI linkers (Biolabs) by standard methods (Maniatis *et al.*, 1982). After digestion with *Eco*RI (Boehringer Mannheim), cDNA of larger size (mean size ~900 bp) was selected and separated from unligated linkers by chromatography on Biogel A 15 m. *Eco*RI-digested and alkaline phosphatase-treated λ gt 11 DNA was a kind gift of G.McMaster. cDNA was ligated to vector arms with T4 DNA ligase (Biolabs) at a molar ratio of ~1:2 and the phages packaged with Packaging Extract (Promega Biotec) following the instructions of the manufacturer. 290 ng of double-stranded cDNA resulted in a library of 8.8×10^5 plaques of which >85% carried inserts.

Antibody screening

The preparation and specificity of mAbs H28, P61 and rabbit anti-N-CAM serum have been described (Hirn *et al.*, 1981; Gennarini *et al.*, 1984a). Before use, the rabbit serum was extensively absorbed with *E. coli* membranes and with *E. coli* lysates coupled to Sepharose. [^{125}I]Iodinated rabbit anti-rat Ig (for mAbs) or [^{125}I]-iodo-protein A were used as radioactive probes. The cDNA library was amplified on *E. coli* strain Y1090 (Young and Davis, 1983b) and screened by a slightly modified version of the plaque screening procedure described by Young and Davis (1983b) and Schwarzbauer *et al.* (1983). Y1090 bacteria were selected for maximum repression of *lacZ* gene expression by isolating the colonies which gave a minimum of blue plaques (<10%) on 5-bromo-4-chloro-3-indolyl-β-D-galactose containing plates in the absence of inducer. Instead of soaking the nitrocellulose filters in IPTG, a 10 mM solution was sprayed over the filters after application to the plates (G.McMaster, personal communication). To lower the background, 0.2% Tween 20 was added to all solutions during incubation and washing of the filters.

Initial screening was done with a mixture of the two mAbs (in the form of 1/25 diluted hybridoma supernatant). Rabbit serum was used at a dilution of 1 to 1000. Candidate positive phages from the first screen were retested in pools of five. When a positive signal was obtained, the original plaques were rescreened and the positives taken through successive rounds of antibody screening at progressively lower densities. In the last rounds of screening the specifically positive mAb was used alone.

Fusion proteins expressed by *E. coli* were analyzed by inducing lysogens in strain Y1089 (Young and Davis, 1983a) and preparing Western blots of the total cellular proteins as described (Rougon *et al.*, 1982).

Subcloning and preparation of labeled probes

DNA from confirmed positive, cloned phages was prepared by the small-scale liquid culture method (Maniatis *et al.*, 1982) and subcloned into the *Eco*RI site of pBR328 (Dugaiczyk *et al.*, 1975). Plasmids were isolated from chloramphenicol-amplified cultures and purified by two centrifugations in CsCl (Davis *et al.*, 1980). Purified plasmid DNA was either labeled directly by nick-translation (Rigby *et al.*, 1977) or the inserts were cut out by digestion with *Eco*RI, purified by two cycles of agarose electrophoresis and labeled by the same method.

Gel blot analysis of DNA and RNA

Samples of cytoplasmic or total cellular RNA or of poly(A)$^+$ RNA were electrophoresed on 0.8% or 1.0% agarose in the presence of formaldehyde (Lehrach *et al.*, 1977). Conditions for transfer to nitrocellulose, hybridization and washing of the filters were those described by Thomas (1980). The filters were routinely washed at 65°C to a stringency of 0.2 x SSC (1 x SSC is 0.15 M NaCl/0.015 M sodium citrate).

For genomic DNA analysis, DNA was prepared, digested with *Eco*RI, separated on 0.8% agarose gels, transferred to nitrocellulose and hybridized as described (Steinmetz *et al.*, 1982). Filters were washed at room temperature to a stringency of 0.1 x SSC. In both RNA and genomic DNA blot analyses, labeled probes with a specific activity of $2-6 \times 10^8$ d.p.m./μg were employed at concentrations of 5×10^6 d.p.m./ml.

Similar conditions as for genomic blots were used for hybridization of nick-translated inserts to phage plaques (Maniatis *et al.*, 1982) and to electrophoretically separated restriction fragments. The labeled probes were used at 10^5 d.p.m./ml and the filters washed with a stringency of 0.3 x SSC at 65°C.

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References

Burke,B. and Warren,G. (1984) *Cell*, **36**, 847-856.
Chuong,C.-M., McClain,D.A., Streit,P. and Edelman,G.M. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4234-4238.
Chuong,C.-M. and Edelman,G.M. (1984) *J. Neurosci.*, **4**, 2354-2368.
Davis,R.W., Botstein,D. and Roth,J.R. (1980) *Advanced Bacterial Genetics*, published by Cold Spring Harbor Laboratory Press, NY.
Dugaiczyk,A., Boyer,H.W. and Goodman,H.M. (1975) *J. Mol. Biol.*, **96**, 171-184.
Edelman,G.M., Hoffman,S., Chuong,C.-M., Thiery,J.-P., Brackenbury,R., Gallin,W.J., Grumet,M., Greenberg,M.E., Hemperly,J.J., Cohen,C. and Cunningham,B.A. (1983) *Cold Spring Harbor Symp. Quant. Biol.*, **48**, 515-526.
Favaloro,J, Treisman,R. and Kamen,R. (1980) *Methods Enzymol.*, **65**, 718-722.
Gennarini,G., Rougon,G., Deagostini-Bazin,H., Hirn,M. and Goridis,C. (1984a) *Eur. J. Biochem.*, **142**, 57-64.
Gennarini,G., Hirn,M., Deagostini-Bazin,H. and Goridis,C. (1984b) *Eur. J. Biochem.*, **142**, 65-73.
Goridis,C., Deagostini-Bazin,H., Hirn,M., Hirsch,M.-R., Rougon,G., Sadoul,R., Langley,O.K., Gombos,G. and Finne,J. (1983) *Cold Spring Harbor Symp. Quant. Biol.*, **48**, 527-538.
Grumet,M., Rutishauser,U. and Edelman,G.M. (1982) *Nature*, **295**, 693-695.
Grumet,M., Hoffman,S. and Edelman,G.M. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 267-271.
Hirn,M., Pierres,M., Deagostini-Bazin,H., Hirsch,M.-R. and Goridis,C. (1981) *Brain Res.*, **214**, 433-439.
Hoffman,S. and Edelman,G.M. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 5762-5766.
Kemp,D.J., Coppel,R.L., Cowman,A.F., Saint,R.B., Brown,G.V. and Anders,R.F. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 3787-3791.
Kruse,J., Mailhammer,R., Wernecke,A., Faissner,A., Sommer,I., Goridis,C. and Schachner,M. (1984) *Nature*, **311**, 153-155.
Lehrach,H., Diamond,D., Wozney,J.M. and Boedtker,H. (1977) *Biochemistry (Wash.)*, **16**, 4743-4751.
Leytus,S.P., Chung,D.W., Kisiel,W., Kurachi,K. and Davie,E.W. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 3699-3702.
Lyles,J.M., Norrild,B. and Bock,E. (1984) *J. Cell Biol.*, **98**, 2077-2081.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*, published by Cold Spring Harbor Laboratory Press, NY.
Murray,B.A., Hemperly,J.J., Gallin,W.J., MacGregor,J.S., Edelman,G.M. and Cunningham,B.A. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 5584-5588.
Rasmussen,S., Ramlau,J., Axelsen,N.A. and Bock,E. (1982) *Scand. J. Immunol.*, **15**, 179-185.
Rigby,P.W.J., Dieckmann,M., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237-251.
Rothbard,J.B., Brackenbury,R., Cunningham,B.A. and Edelman,G.M. (1982) *J. Biol. Chem.*, **257**, 11064-11069.
Rougon,G., Deagostini-Bazin,H., Hirn,M. and Goridis,C. (1982) *EMBO J.*, **1**, 1239-1244.
Rutishauser,U., Thiery,J.P., Brackenbury,R., Sela,B.A. and Edelman,G.M. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 577-581.
Rutishauser,U. (1983) *Cold Spring Harbor Symp. Quant. Biol.*, **48**, 501-514.
Sadoul,R., Hirn,M., Deagostini-Bazin,H., Rougon,G. and Goridis,C. (1983) *Nature*, **304**, 347-349.
Schwarzbauer,J.E., Tamkun,J.W., Lemischka,I.R. and Hynes,R.O. (1983) *Cell*, **35**, 421-431.
Steinmetz,M., Minard,K., Horvath,S., McNicholas,J., Frelinger,J., Wake,C., Long,E., Mach,B. and Hood,L. (1982) *Nature*, **300**, 35-42.
Thiery,J.-P., Duband,J.L., Rutishauser,U. and Edelman,G.M. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 6737-6741.
Thomas,P.S. (1980) *Proc.Natl. Acad. Sci. USA*, **77**, 5201-5205.
Werner,D,. Chemla,Y. and Herzberg,M. (1984) *Anal. Biochem.*, **141**, 329-336.
Young,R.A. and Davis,R.W. (1983a) *Proc. Natl. Acad. Sci. USA*, **80**, 1194-1198.
Young,R.A. and Davis,R.W. (1983b) *Science (Wash.)*, **222**, 778-782.

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